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Synthesis of hybrid anticancer agents based on kinase and histone deacetylase inhibitors†

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Fragments based on the VEGFR2i Semaxanib (SU5416, (vascular endothelial growth factor receptor-2 inhibitor) and the HDACi (histone deacetylase inhibitor) SAHA (suberanilohydroxamic acid) have been merged to form a range of low molecular weight dual action hybrids. Vindication of this approach is provided by SAR, docking studies, *in vitro* cancer cell line and biochemical enzyme inhibition data as well as *in vivo Xenopus* data for the lead molecule (*Z*)-N1-(3-((1*H*-pyrrol-2-yl)methylene)-2-oxoindolin-5-yl)-N8-hydroxyoctanediamide **6**.

Introduction

Cancer treatment often employs combinations, or cocktails, of drugs to reduce resistance and improve efficacy.[1] Histone deacetylase inhibitors (HDACis) are gaining importance in anticancer therapy since they can alter the expression of a host of important proteins and transcription factors.[2,3] Indeed, HDACis such as SAHA or valproate can be effectively combined with a number of anticancer agents, *e.g.* platinum-based, Hsp90, kinase or proteasome inhibitors.[4,5,5,6]

VEGFR2 is secreted by tumour cells under hypoxic stress, leading to angiogenesis. Antiangiogenic approaches include the deployment of VEGFR2is or use of HDACis, which can down-regulate the expression of angiogenesis-critical genes. PTK787/ZK 222584, a VEGFR2i, was shown to have excellent VEGFR2 inhibition alone, yet synergized with the HDACi Dacinostat, reducing angiogenesis, much more markedly than the separate components *in vivo* (Fig. 1).[7]

A relatively new concept makes use of a chimeric, or hybrid, drug where the separate components are linked together in one novel entity.[8] Such an approach may lead to improved synergy and efficacy, lower treatment costs (use of one instead of two drugs), avoid dosing and drug–drug interaction issues as well as generate new intellectual property. Indeed, reports of HDACi-containing hybrid agents are increasing exponentially[9–18]and include the SAHA-tarceva-like dual HDAC-kinase inhibitor **1**,[19] which has entered clinical trials, as well as the SAHA-sunitinib-like hybrid **2** (Fig. 2).[20]

Given our interests in synthesizing SAHA[21,22] and Semaxanib-like molecules,[23] this approach prompted us to consider designing a HDACi/VEGFR2i, dual action hybrid related to **2**, although we were unfamiliar of the latter at the onset of our project. Hence, preliminary attempts in this direction involved the reaction of the amines **3** with acid chlorides **4**, followed by hydroxylamine treatment, affording the hybrids **6** and **7**. We also synthesized the shorter chained compound **8** as well as the different benzamide zinc binding group (ZBG)-containing **9** and **10** (Scheme 1).

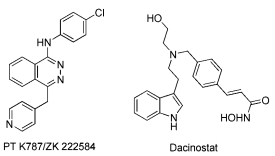

Fig. 1 A VEGFR2i–HDACi combination leads to increased anticancer effects.

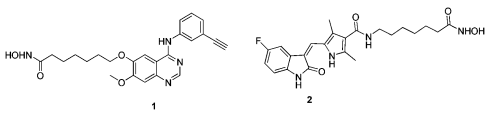

Fig. 2 Examples of hybrid anticancer agents in the prior art.

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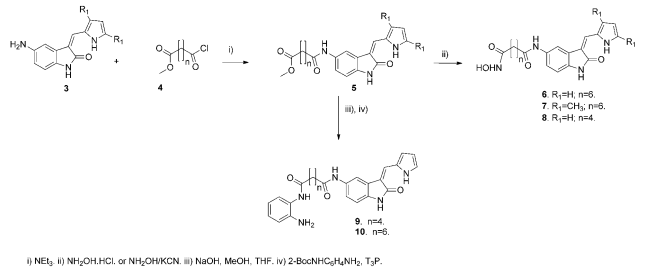
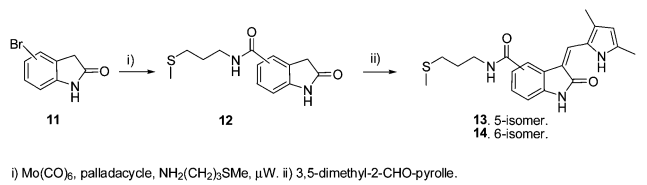
† Electronic supplementary information (ESI) available: Synthesis of hybrids, cell-based assay details. See DOI: 10.1039/c4md00211c



Scheme 1 Synthesis of hybrid molecules.

Initial docking studies predicted the hybrid molecule **6** to display activity towards both kinase and HDAC targets (Fig. 3). Upon binding to HDAC8, the hydroxamate group of **6** was found to coordinate to the enzyme's zinc atom, with the kinase motif being solvent-exposed. However, docking of the hybrid in the VEGFR2 kinase domain is not straightforward, although it is feasible for it to exploit the plasticity of the binding site. When docked into the co-crystal structure of a sunitinib complex (**4agd**), the kinase inhibitor moiety in **6** cannot adopt the conformation observed in the known crystal structure. However, a plausible binding pose is obtained when the hybrid molecule is docked in an alternative VEGFR2 structure (**4asd**), in which the binding pocket has an induced pocket. The latter can accommodate the hydroxamate moiety while still retaining the interactions of the sunitinib moiety. Hybrid **13** comfortably fits in VEGFR2, helping to rationalise its potent kinase activity (*vide infra*).



Scheme 2 Aminocarbonylation routes to hybrids.

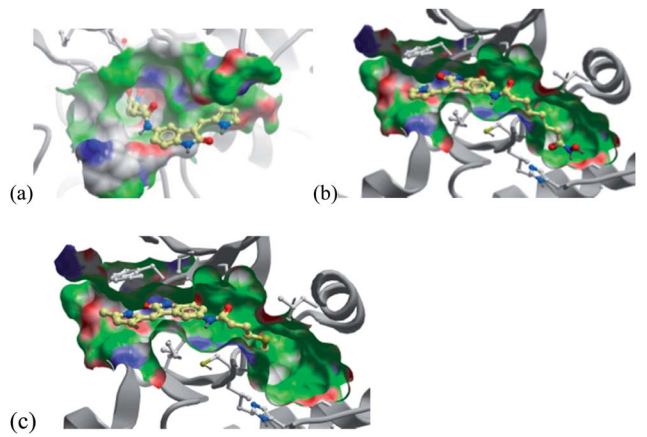


Fig. 3 Docking poses: (a) hybrid **6** in HDAC8 structure (pdb: 1t69): (b) hybrid **6**, (c) hybrid **13** in VEGFR2 kinase structure (pdb: 4asd).

Thioether-containing ZBG analogues were also synthesized (Scheme 2). Hence, the regioisomeric "reverse" amides **13** and **14** were formed by microwave-mediated palladium catalysed aminocarbonylations employing molybdenum hexacarbonyl as a carbon monoxide source (Fig. 3).[24–27]

Both **6** and **7** were tested in biochemical assays against a small number of kinases and compared with the library of hybrids that we synthesized for SAR investigations (Table 1). Hybrid **6** showed poor inhibition of the kinase c-kit when tested at 10 μM concentration yet submicromolar inhibition of three of the four kinases tested (VEGFR1,2, PDGFRa,b).[28,29] Hybrid **7** was significantly less effective. Most of these analogues were less effective than the original hybrid **6**, with the exception of compound **8**, which had improved activity against the two VEGFR isoforms tested. Additionally, the thioether containing hybrid **13** had potent nM kinase activity.

All of the above hybrids were tested *versus* whole cell HDACs at 1 μM concentration using a fluorimetric *in vitro* histone deacetylase assay. Those that exhibited >20% inhibition, *i.e.* **6**–**8**, were tested in a dose-response assay with compound **6** comfortably displaying the best activity, albeit ten-fold less than TSA (Trichostatin A) (Table 2).

Compound **6** also showed excellent HDAC inhibition in a biochemical assay; hybrid **8** had comparable activity to SAHA, which was used as a control, and the benzamides **9** and **10** tended to be significantly less active than SAHA (Table 3). None of the thioether containing hybrids showed any activity and none of the hybrids had significant HDAC4 or HDAC9 activity.[31]

The hybrids were tested *in vivo*, in *Xenopus* embryos, which were exposed to increasing concentrations of compound and assayed. The ability to inhibit HDAC activity was monitored by assaying alpha tubulin acetylation levels, which showed **6** to be

Table 1 Biochemical kinase inhibition data ($n = 2$ unless specified). i.a. = inactive. n.d. = not determined, performed at Reaction Biology

Cpd	IC_{50}; VEGFR1 (nM)	IC_{50}; VEGFR2 (nM)	IC_{50}; PDGFRa (nM)	IC_{50}; PDGFRb (nM)
6	364.5 ± 0.7	237 ± 7	1187 ($n = 1$)	185 ± 13
7	i.a.	i.a.	i.a.	i.a.
8	205 ± 32	290 ± 47	i.a.	518 ($n = 1$)
9	i.a.	i.a.	i.a.	i.a.
10	i.a.	i.a.	i.a.	i.a.
13	i.a.	38 ± 9	11.8 ± 0.2	8.0 ± 0.02
14	i.a.	i.a.	245 ± 15	i.a.
SU5416 (ref. 30)	43 ± 11	220 ± 34	n.d	68 ± 2

Table 2 Cell-based HDAC inhibition studies

Cpd	IC_{50} (nM) with SEM
6	117 ± 29
7	443 ± 15
8	935 ± 29
TSA	12 ± 1

Table 3 Biochemical HDAC inhibition for hybrid molecules

	SAHA		6		8		9		10	
	EC_{50} (M)	SD	EC_{50} (M)	SD	EC_{50} (M)	SD	EC_{50} (M)	SD	EC_{50} (M)	SD
HDAC1	2.24×10^{-8}	2.95×10^{-9}	6.87×10^{-12}	1.13×10^{-11}	9.37×10^{-9}	1.29×10^{-9}	1.26×10^{-6}	1.67×10^{-7}	3.73×10^{-7}	3.50×10^{-8}
HDAC2	5.60×10^{-8}	3.18×10^{-9}	1.11×10^{-9}	4.31×10^{-11}	2.04×10^{-8}	2.76×10^{-9}	3.29×10^{-6}	4.45×10^{-7}	2.23×10^{-6}	3.26×10^{-7}
HDAC3	1.91×10^{-8}	7.07×10^{-10}	6.69×10^{-10}	4.51×10^{-11}	9.77×10^{-9}	1.06×10^{-9}	2.42×10^{-7}	9.69×10^{-9}	3.94×10^{-7}	2.61×10^{-8}
HDAC4	—	—	—	—	—	—	—	—	—	—
HDAC5	—	—	8.50×10^{-7}	1.76×10^{-7}	—	—	—	—	—	—
HDAC6	2.71×10^{-9}	1.35×10^{-10}	3.23×10^{-10}	5.53×10^{-11}	1.61×10^{-9}	1.59×10^{-10}	—	—	4.61×10^{-6}	5.75×10^{-7}
HDAC7	—	—	—	—	—	—	—	—	—	—
HDAC8	1.02×10^{-6}	6.48×10^{-8}	6.02×10^{-8}	1.05×10^{-8}	3.12×10^{-7}	5.28×10^{-8}	—	—	—	—
HDAC9	—	—	—	—	—	—	—	—	—	—

[a] Activity (IC_{50}) in μM. Blank boxes indicate no significant activity. Average of >2 runs.

Fig. 4 Sets of twelve 2-cell *Xenopus laevis* embryos were treated with compound **6** (top) or SAHA (bottom) at the concentrations shown until they developed to stage 14. Protein extracts equivalent to 1 embryo were separated by duplicate SDS-PAGE gels and acetylated alpha-tubulin or total alpha-tubulin were detected by Western blotting.

Fig. 5 Sets of 12 2-cell *X. laevis* embryos, treated with DMSO or increasing μM concentrations of compound **6** at stage 9, allowed to develop to stage 38, fixed and Egfl7 mRNA levels determined by *in situ* hybridisation.

Fig. 6 Dose–response curves for SAHA/**3b** mixture (open circles) and hybrid drug (black circles) exposed for 72 h to MDA-MB231 breast cancer cells. The results are expressed as mean ± s.e.m of triplicate assays.

the most effective deacetylation inhibitor tested of the series, even more effective than SAHA. Hybrid **6** produced a maximal deacetylation inhibition at the lowest concentration tested *i.e.* 10 uM (Fig. 4).

Table 4 NIH cell panel assay for hybrid **6**

Panel	Cell line	GI_{50}[a] (nM)
Leukemia	CCRF-CEM	102
	HL-60(TB)	477
	K-562	251
	MOLT-4	302
	RPMI-8226	2920
	SR	296
NSCL[c]	A549/ATCC	2905
	HOP-62	381
	HOP-92	412
	NCI-H226	2320
	NCI-H23	1320
	NCI-H322M	1017
	NCI-H460	2745
	NCI-H522	87
Colon	COLO 205	248
	HCC-2998	1635
	HCT-116	238
	HCT-15	4590
	HT29	612
	KM12	960
	SW-620	275
CNS	SF-268	1194
	SF-295	1310
	SF-539	605
	SNB-19	644
	SNB-75	194[b]
	U251	420
Melanoma	LOX IMVI	565
	MALME-3M	86
	M14	489
	MDA-MB-435	341
	SK-MEL-2	989
	SK-MEL-28	406
	SK-MEL-5	243
	UACC-257	370
	UACC-62	301
Ovarian	IGROV1	167
	OVCAR-3	3600
	OVCAR-4	1270[b]
	OVCAR-5	1120
	OVCAR-8	499
	NCI/ADR-RES	4000
	SK-OV-3	363
Renal	786-0	2400
	A498	365
	ACHN	1150
	CAKI-1	2330[b]
	RXF 393	518
	SN12C	2190
	TK-10	795
	UO-31	775
Prostate	PC-3	917
	DU-145	1016
Breast	MCF7	440
	MDA-MB-231/ATCC	1000
	HS 578T	1022
	BT-549	1945
	MDA-MB-468	194
	T-47D	45[b]

[a] GI_{50} = concentration to achieve 50% inhibition (National Cancer Institute, NCI); mean value of two runs. [b] One run. [c] Non small cell lung.

By contrast, when its ability to affect vasculogenesis was tested, compound **6** was less effective than its single compound equivalent, Semaxanib. Its major effect was to inhibit Egfl7 expression, a marker for angiogenisis, in the heart in a dose dependent manner. Expression in intersomitic vessels (black arrows, Fig. 5d) was affected to a lesser extent. The failure of the Egfl7-expressing vascular endothelial cells to form tubes is also apparent in the presence of compound **6**; this can be seen when their punctate staining (blue arrow, Fig. 5d) is compared with the uninterrupted tube of the posterior cardinal vein present in control embryos (Fig. 5a). Egfl7 is expressed in vascular endothelial cells and is directly activated by VEGFR2. The red arrow shows the absence of Egfl7 in the heart as concentrations were increased and the black arrow shows its absence from the intersomitic vessels. The blue arrow shows that the endothelial cells that normally form the posterior cardinal vein have failed to form a tube.

We tested the effect on cell viability of dose-dependent incubation for 72 h of MDA-MB231 cells with either a 1 : 1 mixture of SAHA (IC_{50} of SAHA in MDA-MB231 cells is 1.8 μM)[32] and the kinase inhibitor **3b** or the hybrid molecule **6** in a MTT assay.[33] As shown in Fig. 6 the mixture displays a dose–response U-shaped curve with a maximal effect equal to about 10 μM and a reversion to 70% of cell viability at the highest concentration used (*i.e.* 200 μM). Such U-shaped curves (hormesis) have precedence in anticancer assays.[34] On the other hand, the hybrid drug shows a sigmoidal response with an IC_{50} = 29 μM and a reduction of cell survival down to *ca.* 30% *vs.* control at 200 μM concentration.

The hybrids were tested in a NCI single-dose *in vitro* assay, with compound **6** being selected for repeat dosing over a five-dose range (Table 4) where it exhibited generally sub-micromolar GI_{50} values, with notable findings (all duplicate averaged values) including the leukemia cell line, CCRF-CEM (GI_{50} = 102 nM), the NSCL cell line NCI-H522 (GI_{50} = 87 nM) and the melanoma cell line MALME-3M (GI_{50} = 86 nM).

Conclusions

Compound **6** is an effective dual-action hybrid. Its promising preliminary data, notably in biochemical and *in vitro* assays as well as in the NIH cell line, render it an effective potential chemical probe for angiogenisis.[35,36] Current studies are aimed at exploring further SAR in these hybrids with regard to HDAC isoform[37,38] and kinase selectivity[39] as well as improving their physiochemical properties,[40] and will be reported in due course.

Financial disclosure

The patent filing (JS inventor, ref. 35) for this series of compounds is no longer active.

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Notes and references

1 J. E. Dancey and H. X. Chen, *Nat. Rev. Drug Discovery*, 2006, **5**, 649.
2 J. Li, G. Li and W. Xu, *Curr. Med. Chem.*, 2013, **20**, 1858.
3 P. A. Marks and R. Breslow, *Nat. Biotechnol.*, 2007, **25**, 84.
4 R. Nimmanapalli, L. Fuino, C. Stobaugh, V. Richon and K. Bhalla, *Blood*, 2003, **101**, 3236.
5 H. V. Diyabalanage, M. L. Granda and J. M. Hooker, *Cancer Lett.*, 2013, **329**, 1.
6 N. Batty, G. G. Malouf and J. P. Issa, *Cancer Lett.*, 2009, **280**, 192.
7 X. W. David, Z. Qian, S. K. Kachhap, Y. Kato, Y. Wei, L. Zhang, P. Atadja and R. Pili, *Cancer Res.*, 2004, **64**, 6626.
8 S. Fortin and G. Berube, *Expert Opin. Drug Discovery*, 2013, **8**, 1029.
9 T. Beckers, S. Mahboobi, A. Sellmer, M. Winkler, E. Eichhorn, H. Pongratz, T. Maier, T. Ciossek, T. Baer, G. Kelter, H.-H. Fiebig and M. Schmidt, *MedChemComm*, 2012, **3**, 829.
10 K. Liu, D. Zhang, J. Chojnacki, Y. Du, H. Fu, S. Grant and S. Zhang, *Org. Biomol. Chem.*, 2013, **11**, 4757.
11 J. B. Chen, T. R. Chern, T. T. Wei, C. C. Chen, J. H. Lin and J. M. Fang, *J. Med. Chem.*, 2013, **56**, 3645.
12 K. S. Ko, M. E. Steffey, K. R. Brandvold and M. B. Soellner, *ACS Med. Chem. Lett.*, 2013, **4**, 779.
13 Y. Tanaka, K. Aikawa, G. Nishida, M. Homma, S. Sogabe, S. Igaki, Y. Hayano, T. Sameshima, I. Miyahisa, T. Kawamoto, M. Tawada, Y. Imai, M. Inazuka, N. Cho, Y. Imaeda and T. Ishikawa, *J. Med. Chem.*, 2013, **56**, 9635.
14 W. Guerrant, V. Patil, J. C. Canzoneri and A. K. Oyelere, *J. Med. Chem.*, 2012, **55**, 1465.
15 J. Cazares Marinero Jde, M. Lapierre, V. Cavailles, R. Saint-Fort, A. Vessieres, S. Top and G. Jaouen, *Dalton Trans.*, 2013, **42**, 15489.
16 L. W. L. Woo, C. Bubert, A. Purohit and B. V. L. Potter, *ACS Med. Chem. Lett.*, 2011, **2**, 243.
17 S. Mahboobi, A. Sellmer, M. Winkler, E. Eichhorn, H. Pongratz, T. Ciossek, T. Baer, T. Maier and T. Beckers, *J. Med. Chem.*, 2010, **53**, 8546.
18 L. K. Gediya, A. Khandelwal, J. Patel, A. Belosay, G. Sabnis, J. Mehta, P. Purushottamachar and V. C. O. Njar, *J. Med. Chem.*, 2008, **51**, 3895.
19 X. Cai, H. X. Zhai, J. Wang, J. Forrester, H. Qu, L. Yin, C. J. Lai, R. Bao and C. Qian, *J. Med. Chem.*, 2010, **53**, 2000.
20 C. Qian, X. Cai and H. Zhai, *US Pat.*, 2009/0076044A1, 2009.
21 J. Spencer, J. Amin, R. Boddiboyena, G. Packham, B. E. Cavell, S. S. Syed Alwi, R. M. Paranal, T. D. Heightman, M. Wang, B. Marsden, P. Coxhead, M. Guille, G. J. Tizzard, S. J. Coles and J. E. Bradner, *MedChemComm*, 2012, **3**, 61.
22 J. Spencer, J. Amin, M. Wang, G. Packham, S. S. Alwi, G. J. Tizzard, S. J. Coles, R. M. Paranal, J. E. Bradner and T. D. Heightman, *ACS Med. Chem. Lett.*, 2011, **2**, 358.
23 J. Spencer, J. Amin, S. K. Callear, G. J. Tizzard, S. J. Coles, P. Coxhead and M. Guille, *Metallomics*, 2011, **3**, 600.
24 B. Roberts, D. Liptrot, L. Alcaraz, T. Luker and M. J. Stocks, *Org. Lett.*, 2010, **12**, 4280.
25 P. Nordeman, L. R. Odell and M. Larhed, *J. Org. Chem.*, 2012, **77**, 11393.
26 M. Larhed, L. Odell and F. Russo, *Synlett*, 2012, **23**, 685.
27 J. Spencer, R. P. Rathnam, H. Patel and N. Anjum, *Tetrahedron*, 2008, **64**, 10195.
28 L. Sun, C. Liang, S. Shirazian, Y. Zhou, T. Miller, J. Cui, J. Y. Fukuda, J.-Y. Chu, A. Nematalla, X. Wang, H. Chen, A. Sistla, T. C. Luu, F. Tang, J. Wei and C. Tang, *J. Med. Chem.*, 2003, **46**, 1116.
29 L. Sun, N. Tran, F. Tang, H. App, P. Hirth, G. McMahon and C. Tang, *J. Med. Chem.*, 1998, **41**, 2588.
30 R. Roskoski, Jr, *Biochem. Biophys. Res. Commun.*, 2007, **356**, 323.
31 J. E. Bradner, N. West, M. L. Grachan, E. F. Greenberg, S. J. Haggarty, T. Warnow and R. Mazitschek, *Nat. Chem. Biol.*, 2010, **6**, 238.
32 K. E. Joung, K. N. Min, J. Y. An, D. K. Kim, G. Kong and Y. Y. Sheen, *Cancer Res.*, 2006, **66**, 5394.
33 M. Librizzi, A. Longo, R. Chiarelli, J. Amin, J. Spencer and C. Luparello, *Chem. Res. Toxicol.*, 2012, **25**, 2608.
34 F. Donate, G. C. Parry, Y. Shaked, H. Hensley, X. Guan, I. Beck, Z. Tel-Tsur, M. L. Plunkett, M. Manuia, D. E. Shaw, R. S. Kerbel and A. P. Mazar, *Clin. Cancer Res.*, 2008, **14**, 2137.
35 J. Spencer, WO 025726A1, 2012.
36 P. Workman and I. Collins, *Chem. Biol.*, 2010, **17**, 561.
37 S. Balasubramanian, E. Verner and J. J. Buggy, *Cancer Lett.*, 2009, **280**, 211.
38 M. Weïwer, M. C. Lewis, F. F. Wagner and E. B. Holson, *Future Med. Chem.*, 2013, **5**, 1491.
39 R. Kumar, M. C. Crouthamel, D. H. Rominger, R. R. Gontarek, P. J. Tummino, R. A. Levin and A. G. King, *Br. J. Cancer*, 2009, **101**, 1717.
40 S. Schlimme, A. T. Hauser, V. Carafa, R. Heinke, S. Kannan, D. A. Stolfa, S. Cellamare, A. Carotti, L. Altucci, M. Jung and W. Sippl, *ChemMedChem*, 2011, **6**, 1193.